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# Estimating the Potency of Mutagens Cytotoxicity as an Obligatory Consequence of Mutagenicity

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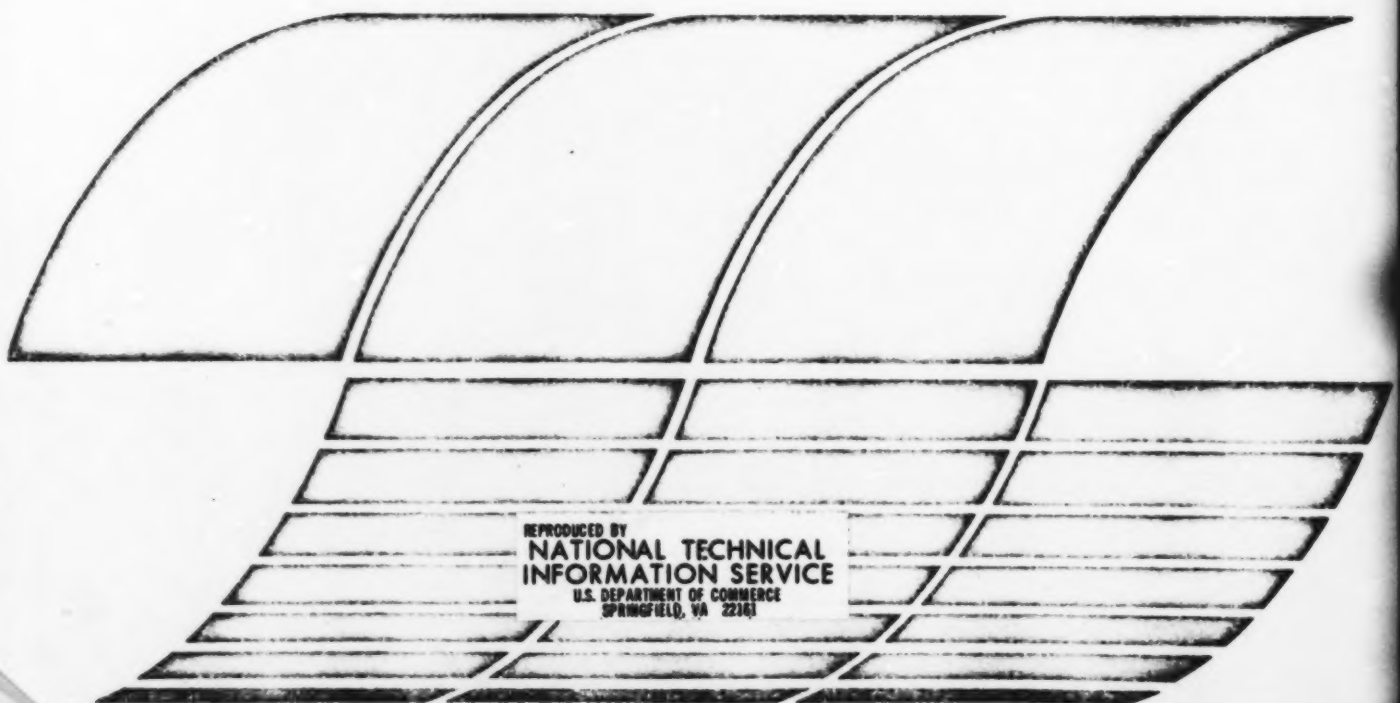
# Estimating the Potency of Mutagens

## Cytotoxicity as an Obligatory Consequence of Mutagenicity

## Interagency Energy/Environment R&D Program Report



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Rapid and reliable screening methods are required for identifying environmental mutagens and estimating their mutagenic potency in preparation for use of more elaborate tests to assess the genetic risk to man. On theoretical grounds, one expects a certain minimum cytotoxic potency to correlate with mutagenic potency, particularly when the latter is measured using forward mutations that result in inactive gene products. Furthermore, as cells cannot divide unless a substantial fraction of their genome is functionally intact, mutagenic agents should also be obligatory cytotoxic agents, with a given mutagenicity conferring a certain irreducible cytotoxicity. We show here that the cytotoxic potency of 22 chemical mutagens is highly correlated with their mutagenic potency as assayed in five rodent and human in vitro cell systems. This relationship implies that the maximum potential mutagenic potency of such compounds may be reliably estimated from rapid and straightforward measurements of their cytotoxic potency, the latter defined as the failure of cultured cells to undergo continued cell division. The estimate is necessarily a maximum one, as an agent may exert cytotoxic effects by pathways indpendent of its mutagenic action.

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## Cytotoxicity as an Obligatory Consequence of Mutagenicity

by

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## FOREWORD

Short-term toxicologic testing, particularly methods for detecting genetically active substances, has become a keystone of the Environmental Protection Agency's efforts to detect and evaluate environmental hazards. Among the most serious hazards to man's future are agents that damage the genome, which is the information bank of cells and organisms, by mechanisms that can increase the future population load of inherited structural defects and genetic diseases or that can increase the incidence of cancer in the current generation. Tests for environmental mutagens play a dual role by virtue of their ability to detect mutagens and their presumptive ability to detect carcinogens, owing to abundant laboratory evidence that most known carcinogens respond positively to tests for mutagens.

The Biomedical Sciences Division of the Lawrence Livermore Laboratory has for the past five years been heavily engaged in the development and application of short-term tests for genetic toxicology. Specific examples are as follows:

- basic studies of classes of mutations in mammalian cells in culture and methods for selective recovery of mutant cells;
- development of suitable strains of cells for detection of forward mutations at multiple loci, and of detailed experimental protocols for mutagen detection;
- development and application of multiple modes of testing *in vitro* and *in vivo* for chromosomal damage and misrepair in the form of sister chromatid exchange, together with simultaneous correlation with mutation induction;
- development and application of highly sensitive tests for injury to sperm cells in the male testis and to oocytes in the juvenile female ovary;
- development of automated cytochemical methods for detection of rare events of mutation and malignant transformation of cells in living animals, including man;
- application of cytogenetic and automated cytopathologic methods to workforce populations engaged with energy technology, and

- in collaboration with our Environmental Sciences Division, application of a battery of the foregoing short-term tests to complex effluents from coal gasification and oil shale retorting technologies.

The following report represents an analysis of quantitative data on cytotoxicity and mutation induction in mammalian cultured cell systems for our laboratory and a review of the literature. The analysis revealed that the cytotoxic potency of 22 chemical mutagens is linearly correlated to a high degree with their mutagenic potency in five rodent and human cell systems. This relationship indicates that the potential mutagenic potency of chemicals can be reliably estimated from assays for their cytotoxicity. Since the latter assays are rapid and relatively inexpensive, they are suggested for wide application to screening of, for example, industrial chemicals for potential genetic toxicity as a precursor to setting priorities for further testing. Thus, a substance exhibiting high toxicity (i.e., killing efficiency) for mammalian cells would be given priority for mutagenesis and other genetic toxicology assays. A substance exhibiting low cytotoxicity could be, at most, a weak mutagen and would have a lower urgency for further testing.

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## ABSTRACT

Rapid and reliable screening methods are required for identifying environmental mutagens and estimating their mutagenic potency in preparation for use of more elaborate tests to assess the genetic risk to man. In this report, we show that the cytotoxic potency of 22 chemical mutagens is highly correlated with their mutagenic potency as assayed in five rodent and human *in vitro* cell systems. This relationship implies that the maximum potential mutagenic potency of such compounds may be reliably estimated from rapid and straight-forward measurements of their cytotoxic potency, the latter defined as the failure of cultured cells to undergo continued cell division.



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## SECTION 1

## INTRODUCTION

A variety of short-term mutagenesis assays employing both procaryotic and eucaryotic systems are being used to identify potentially hazardous agents in the environment. At present, the screening of a large number of compounds is most effectively done with *in vitro* mutagenesis assays, of which the main types use either bacterial or mammalian cells. Although tests with mammalian cells are not as rapid and inexpensive as microbial assays, they are needed to confirm and extend the bacterial results. Indeed, because of known differences in genome organization and possible differences in metabolic and repair processes, mutagenicity assays using mammalian cells may provide a better assessment of potential risk to humans than do microbial tests. It would be desirable to screen all potential mutagens with mammalian cell mutagenicity assays, but this does not seem feasible unless, as we propose, test compounds are first prescreened on the basis of their cytotoxic potency.

## SECTION 2

## DISCUSSION

On theoretical grounds, one expects a certain minimum cytotoxic potency to correlate with mutagenic potency, particularly when the latter is measured using forward mutations that result in inactive gene products. In fact, since cells cannot divide unless a substantial fraction of their genome is functionally intact, mutagenic agents should be obligatory cytotoxic agents as well, with a given mutagenicity conferring a certain irreducible cytotoxicity. Thus, assays of cytotoxic potency, which are relatively easy to perform with mammalian cells, might provide a reliable estimate of an agent's maximum potential mutagenic potency. The estimate is necessarily a maximum one, since an agent may exert cytotoxic effects by pathways independent of its mutagenic action.

A correlation between cell survival and mutagenic response has been noted for ionizing radiation mutagenesis[1] and appears to be applicable to ultraviolet radiation and certain chemicals as well[2-4]. Roberts et al. have discussed the molecular aspects of cellular lethality and mutagenesis in terms of damage to DNA[5]. To further test this relationship, we have compiled data on the cytotoxicity and mutagenicity of the 24 chemical and physical mutagens listed in Table 1. As the measure of cytotoxic potency, we have chosen to use the $D_{37}$ unit of survival, defined here as the dose required to kill approximately 63% of the initial cell population. Unlike $D_0$, estimation of $D_{37}$ is usually possible from the limited survival data that accompanies published mutation frequencies; $D_{37}$ generally measures survival within the linear portion of the dose response for mutation (lower survivals are frequently out of the linear mutation range). Moreover, this parameter reflects differences in repair capability or fidelity that may influence both toxicity and mutagenicity.

Table 1 Compounds evaluated for mutagenic potency

| Compound | n[a] | Ref. |
|---|---|---|
| **Alkylating agents** | | |
| Streptozotocin (SZ) | 1 | 16 |
| Dimethylnitrosamine (DMN) | 2 | 22,30 |
| Ethyl methanesulfonate (EMS) | 15 | 6,10,11,12,14 15,25,27,28,34,41 |
| Diethylsulfate (DES) | 1 | 9 |
| N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) | 13 | 6,8,12,16,17,20 24,26,33,38,39 |
| N-methyl-N-nitrosourea (MNU) | 2 | 8,39 |
| N-ethyl-N-nitrosourea (ENU) | 1 | 8 |
| 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) | 2 | 26,35 |
| N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) | 1 | 8 |
| N-butyl-N-nitrosourea (BNU) | 1 | 8 |
| Methyl methanesulfonate (MMS) | 2 | 9,29 |
| Dimethylsulfate (DMS) | 1 | 9 |
| **Polycyclic aromatic hydrocarbons (PAH)** | | |
| Benzo(a)pyrene, diol epoxide (BPde) | 1 | 23 |
| 3-methylcholanthrene (MC) | 2 | 21 |
| Benzo(a)pyrene (BP) | 3 | 19,21 |
| 7,12-dimethylbenz(a)anthracene (DMBA) | 2 | 21 |
| 7,bromomethylbenz(a)anthracene (MBA-7br) | 1 | 18 |
| 7,12-dimethylbenz(a)anthracene, 5,6-oxide (DMBAox) | 1 | 36 |
| Dibenz(a,h)anthracene, 5,6-oxide (DBAox) | 1 | 36 |
| Benzo(a)pyrene, 4,5-oxide (BPox) | 1 | 36 |
| **Frameshift mutagens** | | |
| Heterocyclic nitrogen mustard compounds (ICR's) | 5 | 13,39 |
| **Physical mutagens** | | |
| X- and -irradiation | 4 | 1,5,7,31,32 |
| Ultraviolet irradiation (UV) | 6 | 10,12,15,25, 37,40 |
| **Miscellaneous** | | |
| 4-nitroquinoline-1-oxide (NQO) | 4 | 6 |

[a]Number of determinations evaluated for each compound (some at multiple genetic loci).

In Fig. 1, we compare the frequency of forward mutations induced per applied dose with the reciprocal of the molar dose required for $D_{37}$ survival in three rodent and two human cell systems[5-41]. As plotted, the data constitute a potency index, extending from weakly mutagenic and less toxic compounds to highly mutagenic agents that are toxic at very low concentrations. The relative increase in cytotoxicity is accompanied by a proportional increase in mutagenicity, i.e., the slope of the regression relationship is $1.03 \pm 0.03$. The observed correlation between cytotoxicity and mutagenicity suggests that a determination of the $D_{37}$ dose of toxic agents can be used to estimate the maximum potential induced mutation frequency. In particular, these data permit the hypothesis that no agents will be found whose location on this graph is significantly above the upper dotted line. Whereas mutagenicity and cytotoxicity separately range over six orders of magnitude, no agent can induce more than approximately one forward mutation at a given locus per one hundred cells surviving at the $D_{37}$ dose. If this hypothesis is valid, this is a biological limit to mutagenic potency that is demonstrated by compounds whose cytotoxicity is due solely to mutational events.

On the other hand, we believe the absence of points below the lower dotted line to be artifactual, representing the bias of reported mutagenesis studies for compounds whose ratio of mutagenicity to cytotoxicity is relatively high. Compounds are known that would graph somewhere in this lower range, e.g., a few derivatives of ICR frameshift mutagens[13], a metabolite of a polycyclic hydrocarbon[36], and an inhibitor of DNA synthesis[42] are highly toxic, but apparently are nonmutagenic in mammalian in vitro test systems. Also, certain chemicals yield multiphasic cell survival curves that cast doubt on the $D_{37}$ concept for these mutagens. The shaded area in Fig. 1 reflects values below the detection limit for the systems studied. The cytotoxicity data for non-mutagens would also fall within this range. Thus, the dynamic range for forward mutations in mammalian in vitro systems is approximately four orders of magnitude (from the lowest detectable level to the highest possible mutagenic potency at the $D_{37}$ level of cytotoxicity). Compounds near the bottom of this range will presumably be of greater concern as cytotoxins than as mutagens.

The relative mutagenicity of different compounds at a given cytotoxicity (Fig. 1) is not significantly related to the class of mutagen or the forward mutation marker scored. This is shown in Fig. 2, which displays the observed values of the mutation frequency per locus at the $D_{37}$ dose for different classes of mutagens at different genetic loci. This variation is also not due to systematic differences among cell systems (data not shown).

Cytotoxicity, as defined (Fig. 1), may result from genetic (i.e., DNA-related) and nongenetic mechanisms of injury. Agents that induce the highest mutation frequencies at the $D_{37}$ dose may have the tightest coupling between genetic injury and lethality, i.e., the cytotoxicity related to DNA damage predominates over other forms of general toxicity. Agents inducing lower mutation frequencies at the $D_{37}$ dose may produce a larger proportion of non-genetic injury.

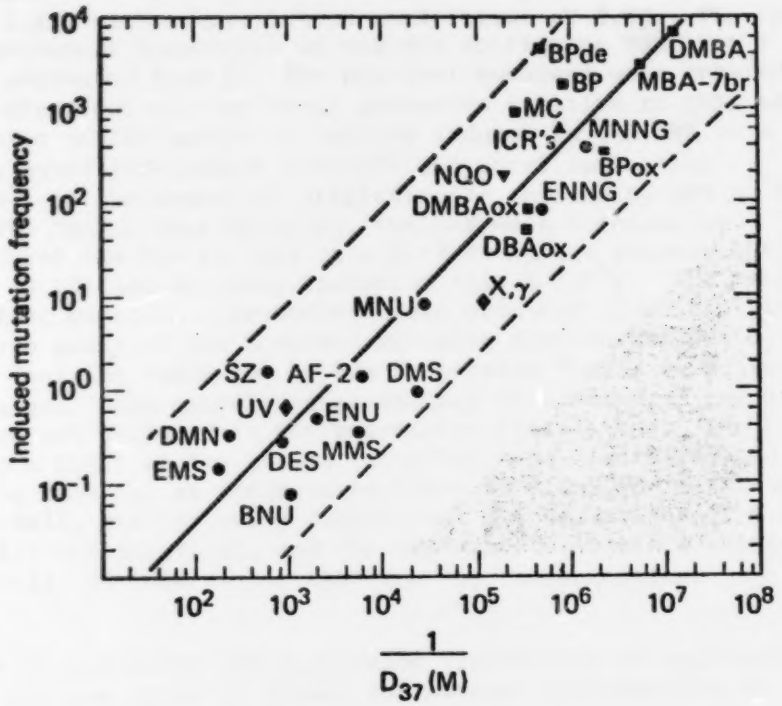


Fig. 1. The relationship between induced mutation frequency per viable cell (M.F). per unit exposure dose (M, mole liter$^{-1}$) and the reciprocal of $D_{37}$ (M), i.e., the dose required to kill 63% of the initial cell population. Killing is defined as the inability of a cell to undergo continued cell division in vitro resulting in a viable cell colony. The $D_{37}$ values were estimated from (1) authors' precise survival responses; (2) computer fits in ref. 14; (3) published tabular data (occasionally over limited dose ranges). In some cases, the values are approximated but individual estimates generally vary no more than ± 20% and never more than ± 50% (in two cases). Mutation frequency data usually included at least two dose points in the most linear range, except for a few reports having only limited data where a single dose and zero dose, i.e., spontaneous frequency, were used. Data included in this plot are those for mutations at the hgprt locus (hypoxanthine-guanine phosphoribosyltransferase, EC 2.4.2.7), aprt locus (adenine phosphoribosyltransferase, EC 2.4.2.8), and the tk locus (thymidine kinase, EC 2.7.1.75). Results in Chinese hamster ovary cells[6-13] (n = 25), V79 hamster cells[14-26] (n = 18), L5178Y mouse lymphoma cells[27-30] (n = 4), normal human diploid fibroblasts[31-37] (n = 9), and human lymphoblasts[38-39] (n = 4) were combined and expressed as the induced frequency of mutants resistant to azaguanine, $AG^r$ (n = 27); thioguanine, $TG^r$ (n = 27); azaadenine, $AA^r$ (n = 4); and bromodeoxyuridine, $BUdR^r$ (n = 2).

Abbreviations for the mutagens are given in Table 1. The linear regression line is given by the equation (log M.F./M) = 1.03 (- log $D_{37}$) - 3.49, with a correlation coefficient of 0.93. The dotted lines represent boundaries of the 95% confidence band for Y data points estimated from X. The physical mutagens were entered on the figure after calculating their potencies relative to EMS, based on the ratios of the number of lesions induced in the DNA of a cell at the $D_{37}$ doses (UV-induced dimers[40] and total ionization lesions[5]) to the number of alkylations[41] induced by EMS at its $D_{37}$. The "molar equivalent $D_{37}$" values were obtained by multiplying the $D_{37}$ for EMS ($6 \times 10^{-3}$ M) by the potency ratios for UV (0.18) and ionizing radiation ($1.4 \times 10^{-3}$). The "molar equivalent mutation frequencies" were obtained by multiplying the geometric means of the observed mutation frequencies per $D_{37}$ for UV and ionizing radiation by the respective "molar equivalent $D_{37}$" values. The calculated parameters were added to the graph, but were not included in the regression calculation. The lower detection limit of the assays (stippled area) was estimated, assuming that (a) at frequencies below $10^{-5}$ induced mutations per viable cell, statistically-significant determinations of M.F. are generally not practical, and (b) the maximum feasible exposure dose is usually 10 fold higher than the $D_{37}$ concentration.

The remarkable similarity among mutation frequencies at equitoxic concentrations has been noted by others for several mutagens[5,16,36]. The correlation between cytotoxicity and mutagenicity need not be due solely to the same type of cellular damage causing both lethal and mutational events[4]; factors that influence the effective exposure of the cells to chemical mutagens (toxification, detoxification), may affect both end points proportionally.

Additional data are needed to establish that partitioning of genetic and nongenetic toxicity induced by a mutagenic agent can be estimated from the magnitude of mutation induced at the $D_{37}$ dose. However, the data in Fig. 1 indicate that cytotoxicity as measured by the $D_{37}$ dosage provides a quantitative estimate of <u>potential</u> mutagenicity. Where appropriate, more specific bioassays can then be applied to determine precisely the <u>in vitro</u> mutagenic potency.

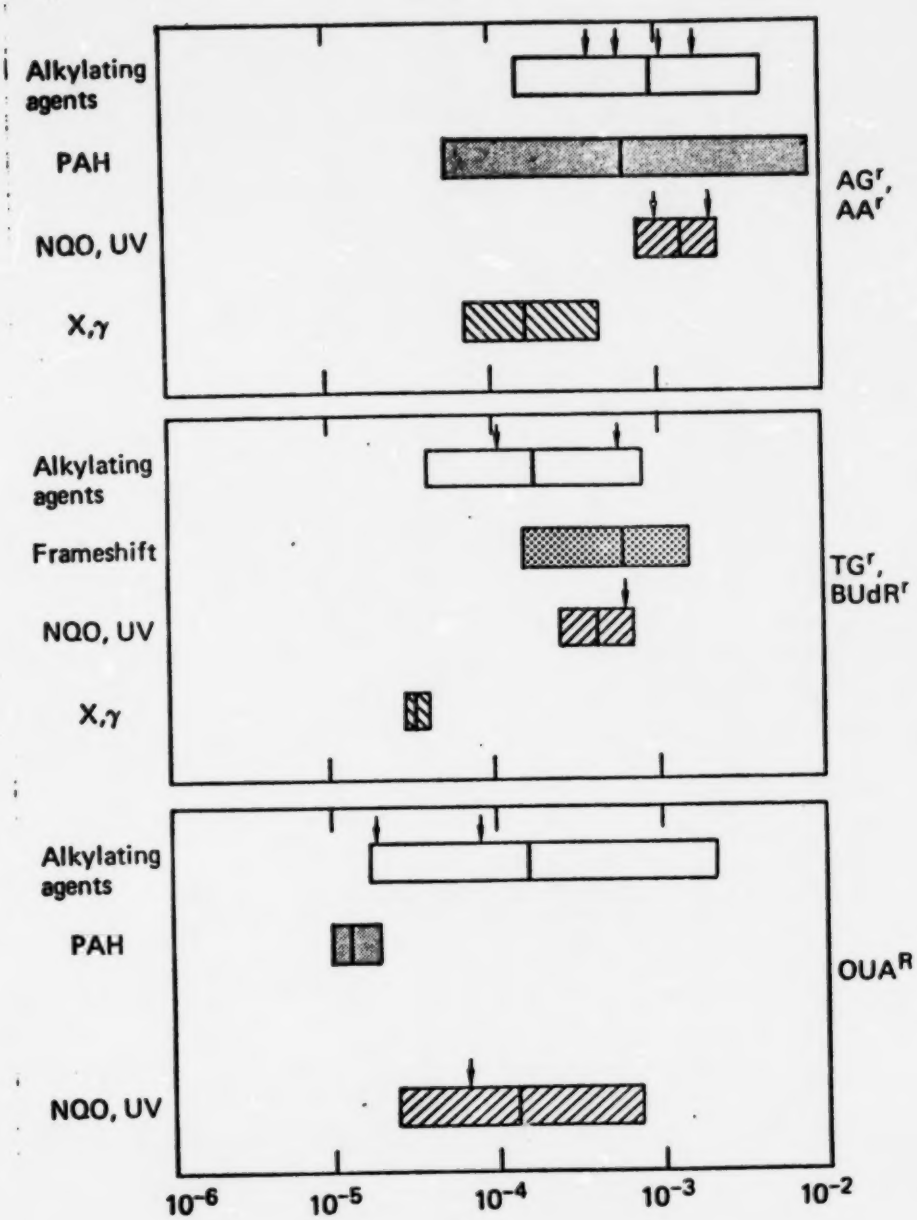


**Fig. 2.** The induced mutation frequency per viable cell (M.F.) calculated for the $D_{37}$ exposure dose of the physical and chemical mutagens

listed in Table 1. The loci include hgprt ($AG^r$,$TG^r$), aprt, ($AA^r$), and tk ($BUdR^r$), as well as resistance to ouabain ($OUA^R$) involving presumed lesions in the Na+-K+-ATPase enzyme (EC 3.6.1.3). The inner mark within bars represents the geometric mean of data for all systems, with the range of reported values indicated by the ends of each bar. The arrows refer to the authors' data for the Chinese hamster ovary multiple marker system[5]. According to the assumptions listed in Fig. 1, the lower limit for determining the M.F. at the $D_{37}$ would be $10^{-6}$.

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